Food Bioscience 63 (2025) 105596

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Effects of a green seaweed from the Atlantic coast (*Ulva lactuca)* on gut microbiota, using an *in vitro* colon model

Aroa López-Santamarina, Paula Roade-Pérez, Alicia del Carmen Mondragón-Portocarrero, Alejandra Cardelle-Cobas *, Alberto Cepeda, José Manuel Miranda

Laboratorio de Higiene Inspección y Control de Alimentos, Departamento de Química Analítica, Nutrición y Bromatología, Universidade de Santiago de Compostela, Campus Terra, Lugo, 27002, Spain

ARTICLE INFO

Keywords:
Ulva lactuca
Colonic fermentation
Bifidobacterium
In vitro
Microbiota modulation

ABSTRACT

Recently, it has been seen that carbohydrates from seaweeds can modify the composition of the gut microbiota and stimulate the production of short chain fatty acids. Seaweed as an interesting alternative to animal protein and possesses, in addition to these carbohydrates, other bioactive components of interest. For this reason, we evaluated the effects of the whole green seaweed *Ulva lactuca* on the intestinal with *in vitro* colonic simulation. Proximate analysis showed that *U. lactuca* is rich in fibre and calcium. In addition, *in vitro* colonic fermentation of this seaweed indicated that it could be used as a carbon source by the gut microbiota; it did not show significant differences in amplicon sequence variants compared with inulin fermentation. Compared with the negative control, the predominant bacterial species after fermentation of *U. lactuca* were *Parabacteroides distasonis* and *Bacteroides ovatus*. Moreover, although to a lesser extent than did inulin, fermentation of *U. lactuca* stimulated the production of short-chain fatty acids. Regarding to the metabolic pathways, statistical differences were found among assays, founding 11 pathways increased for the assays with *U. lactuca* (e.g. PWY-5507 or TOMCAT-PWY, among other 8 and 2 for those developed with inulin (PWY-5304 and PWY-6572). Although more studies are necessaries to confirm the positive effect of *U. lactuca* on the human gut microbiota, this study showed that it has a similar behaviour to inulin, opening new opportunities to investigate the effects of consumption of this whole seaweed in the human health.

1. Introduction

A gut microbiota (GM) that is stable, rich, and diverse in composition and functionality is essential for the development of a host organism and, specifically, to support its health (Rolhion & Chassaing, 2016; Rowland et al., 2018). In contrast, a dysbiotic state leads to detrimental impacts on host health (Degruttola et al., 2016). Non-digestible carbohydrates from the diet can maintain a stable GM in a variety of ways. In fact, one such beneficial effect is the ability to modify the composition of the GM by stimulating the production of secondary metabolites, such as short-chain fatty acids (SCFAs) (Chen et al., 2018). SCFAs are essential for regulating energy homeostasis, releasing gut hormones, improving cholesterol metabolism, and reducing inflammation, among other processes, thus helping to maintain host health (Fang et al., 2019). In addition, the response of the GM to non-digestible carbohydrates can vary depending on the specific composition of these carbohydrates (linkages, monosaccharide composition, molecular weight, etc.), which influences host health (Zhang et al., 2020).

Today, most of the non-digestible polysaccharides in the Western diet are derived from the cell walls of terrestrial plants (López-Santamarina et al., 2020). However, due to intense population growth worldwide in recent decades, freshwater, an essential commodity for agriculture, is becoming increasingly scarce (Cosgrove & Loucks, 2015). According to the Food and Agriculture Organization of the United Nations (FAO, 2009), global agricultural productivity is declining at a rate of about 1% per year. Due to this decline, the demand for food from other sources is expected to increase in the coming years. An alternative to terrestrial vegetables as a source of dietary fibre could be seaweed. Seaweed cultivation has numerous advantages compared to terrestrial plants as it has a fast growth rate and does not require arable land, fresh water or fertilizers (Charoensiddhi et al., 2016). An increase in seaweed cultivation, in addition to food production *per se*, would also

* Corresponding author. Laboratorio de Higiene Inspección y Control de Alimentos. Facultad de Veterinaria pabellón 4 p.b. Campus Universitario 27002-Lugo. Spain.
E-mail address: alejandra.cardelle@usc.es (A. Cardelle-Cobas).

https://doi.org/10.1016/j.fbio.2024.105596
Received 18 September 2024; Received in revised form 22 November 2024; Accepted 30 November 2024
Available online 30 November 2024

Table 1
Comparison of the nutritional composition (g/100g) and mineral content (mg/kg) of *Ulva lactuca* raw and after *in vitro* upper intestinal digestion.

Nutritional composition (% DW matter)	*Ulva lactuca*	
	Raw	After upper *in vitro* digestion
Moisture	$12.88^{a} \pm 0.78$	$<0.5^{b}$
Protein	8.73 ± 0.80	9.16 ± 0.59
Fat	0.51 ± 0.07	0.49 ± 0.04
Carbohydrates	$58.57^{b} \pm 0.87$	$71.46^{a} \pm 1.1$
Sugars	$24.12^{a} \pm 0.65$	$19.22^{b} \pm 0.45$
Dietary fiber	$34.45^{b} \pm 0.87$	$52.24^{a} \pm 0.91$
Ash	$19.31^{a} \pm 0.54$	$11.87^{b} \pm 0.06$
Caloric content (kcal/100g)	204.80 ± 1.45	220.78 ± 2.01
Minerals (mg/kg)		
Ca	$8627.16^{b} \pm 63.57$	$12{,}007.43^{a} \pm 159.5$
Fe	$450.79^{b} \pm 24.71$	$626.96^{a} \pm 16.6$
Cu	4.75 ± 0.5	4.84 ± 1.01
Zn	$9.29^{b} \pm 0.02$	$22.2^{a} \pm 0.13$
As	1.38 ± 0.3	0.93 ± 0.01
Cd	0.06 ± 0.005	0.10 ± 0.001
Hg	<0.05	<0.05
Pb	$0.32^{b} \pm 0.01$	$1.34^{a} \pm 0.02$
I	0.12 ± 0.004	0.08 ± 0.002

Mean ± standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$).

bring environmental benefits, such as CO_2 fixation (Förster and Radulovich, 2016).

Green seaweed remains underutilised in nutraceuticals and pharmaceuticals, despite being important sources of bioactive compounds (Ma et al., 2023); however, they can be found in supermarkets for human consumption. These green seaweeds are rich in protein and poor in fat. In addition, they possess diverse compounds, including vitamins (water- and fat-soluble), minerals (Na, Ca, P, Mg, K, Fe, Zn, Mn, and Cu), and phytochemicals, which makes them interesting alternatives to other foods (Biancarosa et al., 2018; Ma et al., 2023). Moreover, other potential bioactive substances, such as polyphenols, are highly abundant in green seaweeds (Lozano Muñoz & Díaz, 2020), with a content of 1%–5% on a dry-weight basis (Poole et al., 2019; Wekre et al., 2019). Polyphenols are well known for their antioxidant and antimicrobial properties (El-Beltagi et al., 2022; Mahendran et al., 2021), but recent research has indicated that these polyphenols are not fully absorbed in the small intestine and so reach the colon. There, they exert a positive effect on the GM (Gade & Kumar, 2023). The available studies investigating the effects of green seaweeds on the GM have been *in vitro*, most commonly using the genera *Enteromorpha* and *Ulva*, the most abundant genera of green seaweeds (Lopez-Santamarina et al., 2023). Ulvans are the main group of polysaccharides in these species. *Ulva lactuca* is one of the most commercialised *Ulva* species (Miao et al., 2020). Most studies carried out *in vitro* have resulted in increased production of SCFAs; increased growth of beneficial bacterial genera, such as *Lactobacillus*, *Bifidobacterium* (Ajanth Praveen et al., 2019; Kansandee et al., 2024), and *Akkermansia* (Shang et al., 2018); and growth inhibition of potentially pathogenic bacteria (Ren et al., 2017; Seong et al., 2019; Zmora et al., 2019). However, other studies reported no effects on the composition or function of the GM (Kong et al., 2016; Yan et al., 2019; Zhang et al., 2023). Only one of the available studies on these green seaweeds used the whole seaweed plant; the rest used purified polysaccharides that had been purchased or extracted previously in the laboratory (Shang et al., 2018). Given that green seaweed contains compounds other than carbohydrates that can influence the GM, it would be interesting to investigate the effects of whole seaweeds on the GM and consequently on human health. Since the effect on the intestinal microbiota of polysaccharides extracted from seaweed is usually

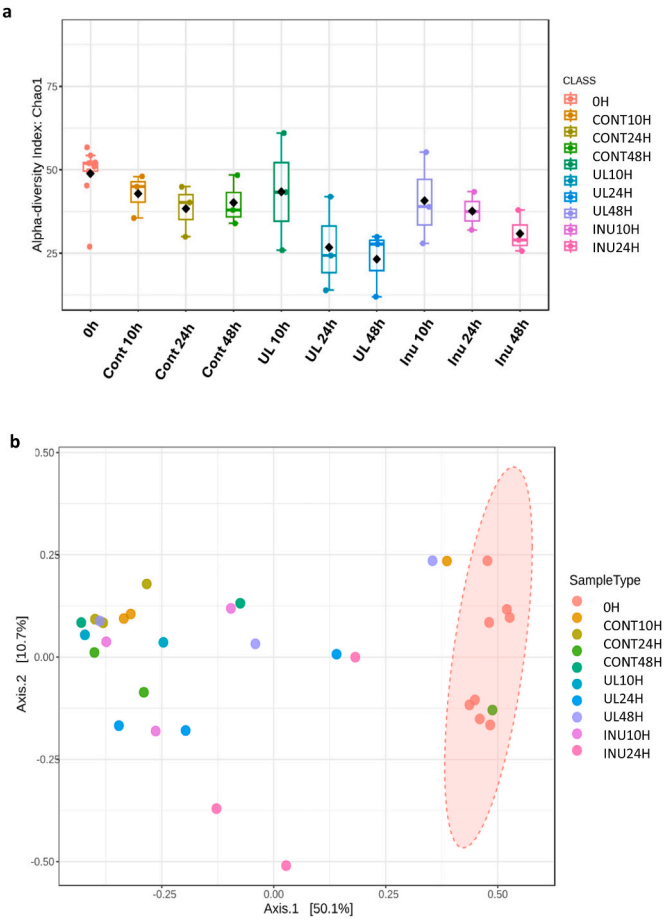

Fig. 1. α- and β-diversity Analysis. Evolution over time of (a) α-diversity (Shannon index) and (b) β-diversity (Bray-Curtis index) in terms of operational taxonomic units for the different samples used (no carbon source-control, inulin, and *U. lactuca*). INU: inulin; CONT: negative control (no substrate addition); UL: *U. lactuca*; 0 h: bacterial composition before substrate addition.

studied, the objective and novelty of this work is to evaluate the effect of the whole matrix of *U. lactuca* on the intestinal microbiota. Thus, taking advantage of other bioactive compounds present in the seaweed that can also be beneficial for the microbiota and human health.

2. Materials and methods

2.1. Seaweed

This study used *U. lactuca* (Portomuiños, Cerceda, A Coruña, Spain). Crushed seaweed (250 g) was freeze-dried using a Labconco™ 77,560-LYPM-LOCK6 apparatus (Kansas City, MO, USA). Subsequently, the seaweed was stored in a desiccator at room temperature (approximately 20 °C) for 1 week prior to analysis of its nutritional composition, the *in vitro* fermentation and SCFAs in the extracts obtained during fermentation.

2.2. Nutritional composition of Ulva lactuca

Nutritional composition analysis was conducted following official methodologies (AOAC, 2022) and in the same way as a previous study (Lopez-Santamarina et al., 2022).The assays were conducted in triplicate and compared with the samples blanks. All nutritional components, except for the percentage of moisture, the sodium content and the caloric content, are expressed as g/100 g of dry matter.

Regard to mineral content (expressed as mg/kg of seaweed), ^{75}As,

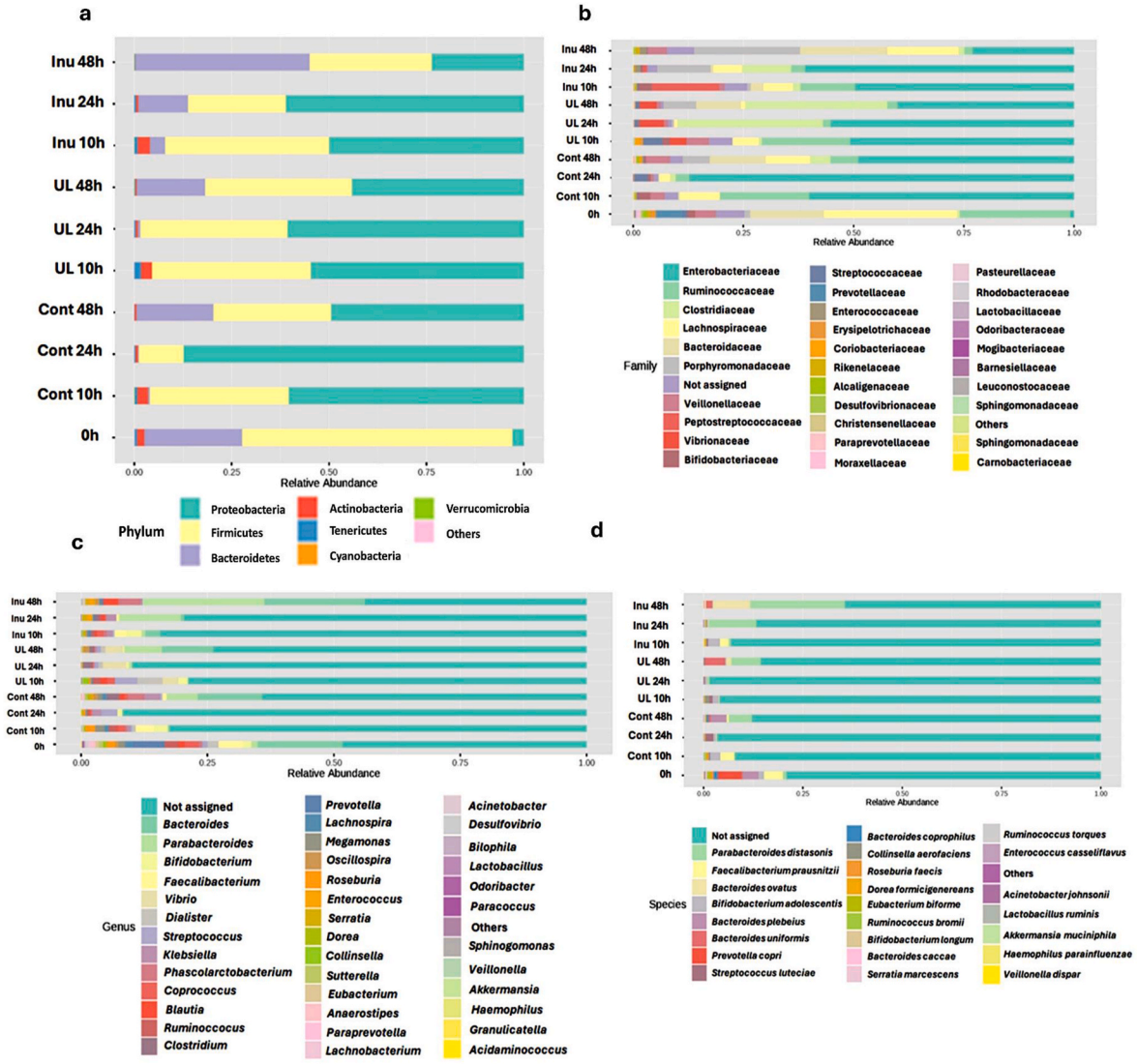

Fig. 2. Analysis of Relative Bacterial Abundance During Substrate Fermentation. Relative abundance (%) of different bacterial phyla (a), family (b), genus (c) and species (d) determined using 16S rRNA amplicon sequencing. The graphic shows the different substrates evaluated at different times (x-axis). INU: inulin; CONT: negative control (no substrate addition); UL: *U. lactuca* and 0h: bacterial composition before substrate addition.

^{43}Ca, ^{112}Cd, ^{63}Cu, ^{56}Fe, ^{202}Hg, ^{127}I, ^{208}Pb and ^{66}Zn were analysed as described previously(Lopez-Santamarina et al., 2022).

Seaweeds must meet safety regulations in terms of toxicological and bacteriological criteria (Rasyid, 2017).

2.3. *In vitro simulation of digestion*

Simulation of the digestion of the upper gastrointestinal tract (oral, gastric, and small intestinal digestion) was performed following the INFOGEST consensus protocol (Brodkorb et al., 2019). The assays were performed in triplicate with 10 g of seaweed. Digested seaweed solutions were cooled to stop the enzymatic reactions and simulation of small intestinal absorption, after the digestion process, was conducted by dialysis at 4 °C as described previously (Lopez-Santamarina et al., 2022). All reagents used in this process were obtained from Sigma-Aldrich. (St. Louis, MO, USA).

2.4. *Donors, stool samples and in vitro colonic simulation*

Faecal samples were donated by two men and one woman between the ages of 32 and 50 years and were recruited through a trial authorized by the Regional Ethics Committee for Clinical Research (Galician Health Service, SERGAS, n° 2018/270). Based on the eligibility criteria of that trial, individuals without gastrointestinal disorders, without chronic pharmacological treatment and who had not consumed antibiotics or pre/pro/postbiotic supplements within 6 months prior to sample collection were included. The volunteers agreed to participate in the study by signing an informed consent form that detailed the utilization of their samples. They were provided with sterile containers to collect stool samples at home. Once collected at home, the sample was delivered to the laboratory within the 2 h, for processing. In the laboratory, the stool samples were diluted 1:10 with phosphate-buffered saline and used immediately for the *in vitro* colonic simulation assays.

In vitro simulation of human colonic digestion was performed based on a published study (Lopez-Santamarina et al., 2022) to evaluate the potential use of seaweed as carbon source by the GM without the presence of other carbon source in the basal medium. A negative control, without carbon source, was carried out simultaneously. Conditions resembling those of the human distal colon, including an anaerobic atmosphere, a temperature of 37 °C and a pH of 6.8, were replicated. The sterilized seaweed substrates were dissolved in the autoclaved basal medium to a final concentration of 1% (*w/v*). Subsequently, 10% (*v/v*) of

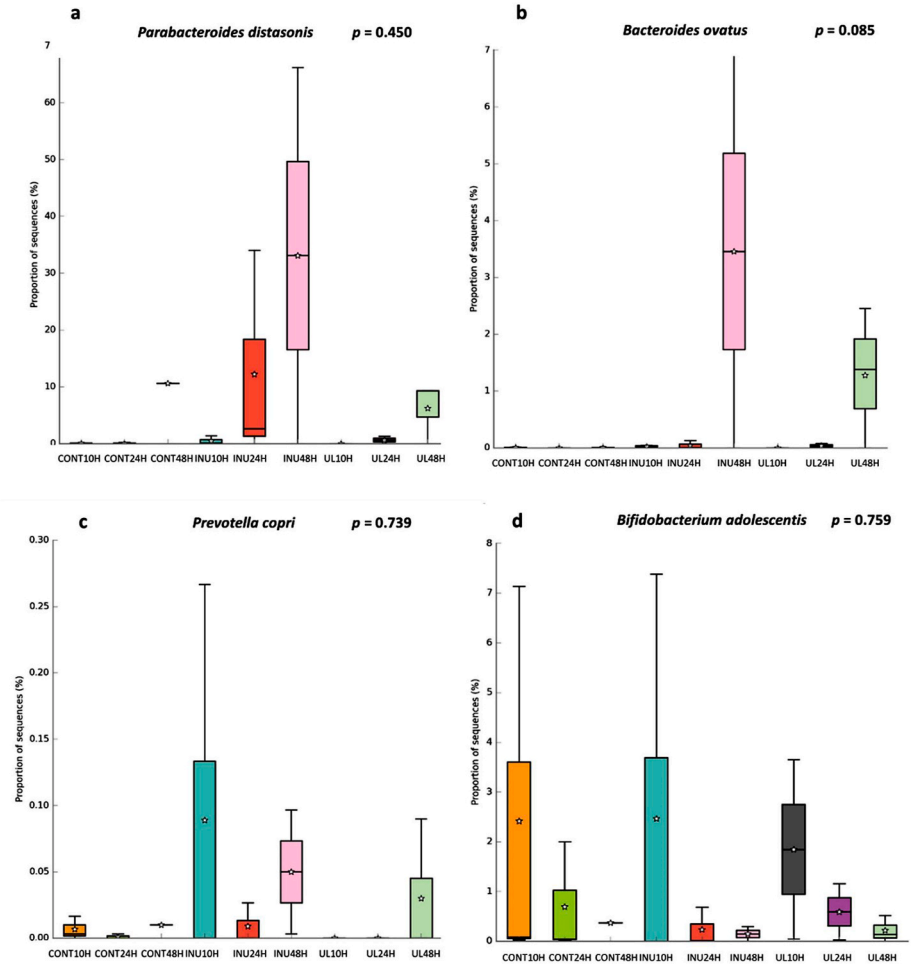

Fig. 3. Bacterial Abundance Profiles of Key Species During Fermentation. Bar plots illustratingthe relative abundance for (a) *Parabacteroides distasonis*, (b) *Bacteroides ovatus,* (c) *Prevotella copri* and (d) *Bifidobacterium adolescentis*. CONT: negative control; INU: inulin; UL: *U. lactuca.*

the previously diluted faeces was inoculated into the vessels. The assays were performed for 48 h; 15 mL samples were taken for analysis at 0, 10, 24 and 48 h of fermentation.

2.5. DNA extraction and 16S ribosomal RNA amplicon sequencing

A portion of the samples taken from the *in vitro* colonic assays were submitted to bacterial DNA extraction and subsequent 16S ribosomal RNA (rRNA) amplicon sequencing, which performed as described in a previous study (Lopez-Santamarina et al., 2023).

2.6. Short-chain fatty-acid analysis

A portion of the samples taken at the different times from the fermentation assays were centrifuged and filtered to analyse the supernatant in terms of SCFAs composition. Samples (1 mL) obtained after fermentation for 0, 10, 24, and 48 h were centrifuged for 10 min at 6100 *g*. The supernatants were removed and filtered through 0.2-μm cellulose acetate membranes (Phenomenex, Torrance, CA, USA). Then, 50 μL of each sample was injected into a Rezex™ ROA-Organic Acid H+ (8%) column (LC Column 300 mm × 7.8 mm; Phenomenex, Torrance, CA, USA) operating at a constant temperature of 50 °C. The mobile phase, 5 mM sulfuric acid, was flashed to the column at a flow rate of 0.5 mL/min in isocratic mode. Analysis was performed with a high-performance liquid chromatography with Diode Array detection (HPLC-PDA) system from Agilent (Waldbronn, Germany) consisting of a binary pump, a degasser, an autosampler, and a column heater coupled to a detector (Infinity 1260 II Diode Array Detector HS; Agilent). Organic acids (lactic, acetic, butyric, propionic, isobutyric, valeric, and isovaleric acids) were obtained from Sigma (Poole, Dorset, United Kingdom). All of them have a purity of 99%, except isovaleric (98%). The SCFAs content was determined by comparing their retention times with standards and quantified by employing the regression formula obtained by plotting different concentrations of the fatty acid against its corresponding area. SCFAs were quantified using the external standard method.

2.7. Statistical and bioinformatic analysis

Statistical analysis of nutritional and mineral composition was performed using a paired Student's *t-test* while analysis of variance (ANOVA) and Tukey's *post-hoc* test were used to establish differences in the results obtained in the SCFAs analysis. All these analyses were performed with SPSS® for Windows v.22 (SPSS Inc., Chicago, IL, USA). Results was considered significant when $p < 0.05$.

For 16S rRNA amplicon sequencing analysis, raw sequencing reads were download from Torrent Suite software (v.5.12.2). These files were processed with Quantitative Insights Into Microbial Ecology (QIIME 2) software v.2023.9 and with MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/). Amplicon sequence variants (ASVs) were produced by using the DADA2 method for quality filtration (Q score >20), trimming, denoising and dereplication. Samples with features (taxa) with a total abundance of <20 were normalized by rarefaction. Taxonomy was assigned to ASVs against the Greengenes 13_8 99% operational taxonomic unit (OTU) reference sequences. Samples were rarefied to a

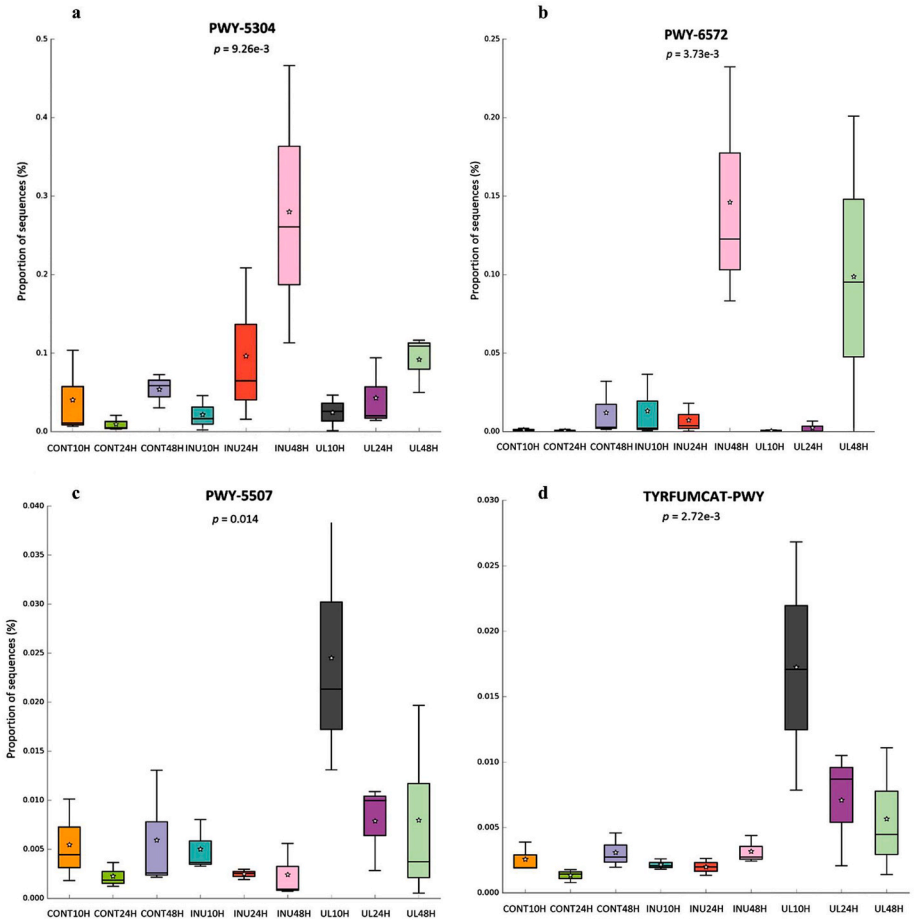

Fig. 4. Significant Differences in Metabolic Pathways. Barplots obtained for PWY-5304, PWY-6572, P381-PWY, PWY-5507 metabolic pathways obtained with PICRUSt. The *p* values obtained show statistical differences among samples. UL: *U. lactuca*, INU: inulin.

Table 2
Metabolic pathways obtained with statistical differences ($p < 0.05$) for assays developed with inulin, *U. lactuca* and no carbon source (negative control).

pathways		*p*-values
CRNFORCAT-PWY	creatinine degradation	0.0020
DENITRIFICATION-PWY	nitrate reduction I (denitrification)	0.0207
LIPASYN-PWY	phospholipases	0.0036
P381-PWY	adenosylcobalamin biosynthesis II (aerobic)	0.0016
PWY-181	photorespiration	0.0148
PWY-5304	superpathway of sulfur oxidation (*Acidianus ambivalens*)	0.0093
PWY-5507	adenosylcobalamin biosynthesis I (anaerobic)	0.0142
PWY-6572	chondroitin sulfate degradation I (bacterial)	0.0037
PWY-7185	UTP and CTP dephosphorylation I	0.0244
PWY-7376	cob(II)yrinate a,c-diamide biosynthesis II (late cobalt incorporation)	0.0110
PWY-7616	methanol oxidation to carbon dioxide	0.0482
TYRFUMCAT-PWY	tyrosine degradation	0.0027

sequencing depth of 30,000 reads and alpha (α) and beta (β)-diversity were determined.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) online Huttenhower Lab Galaxy Server 2.0 was used to predict the functional content of the metagenome. PICRUSt uses an OTU table constructed with the closed benchmark method using QIIME2. The closed benchmark method compares each representative OTU sequence with the reference sequences available in each database, in this study, the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Douglas et al., 2020).

The STAMP software (v 2.1.3; Statistical analysis of taxonomic and functional profiles) was used to perform ANOVA with the Tukey-Kramer *post-hoc* test to determine significant differences between ASVs and metabolic pathways obtained with QIIME2 and PICRUSt, respectively (Parks et al., 2014).

3. Results and discussion

3.1. Nutritional and mineral composition of Ulva lactuca

In terms of nutritional composition, this study analysed the moisture, protein, fat, total carbohydrate, dietary fibre, sugars, ash, and caloric content of the raw seaweed and after simulating digestion in the upper gastrointestinal tract. The mineral content was also analysed, specifically calcium, iron, copper, zinc, arsenic, cadmium, mercury, lead and iodine.

The nutritional composition of *U. lactuca* are shown in Table 1. It is similar to what has been reported in other studies involving *U. lactuca* (Debbarma et al., 2016; Rasyid, 2017). However, it is important to keep in mind that different environmental factors, such as water temperature and salinity, can affect the nutritional composition of seaweeds (Torres et al., 2019). There was a low lipid content and a high protein, carbohydrates, and dietary fibre contents. The protein content was 8.73%,

Table 3
Short-chain fatty-acid (SCFA) concentrations (mM) in the samples obtained for each substrate and each time of colonic fermentation.

SCFAs	0h	Negative control			*Ulva lactuca*			Inulin		
		10h	24h	48h	10h	24h	48h	10h	24h	48h
Lactic	12.98^{a} ± 3.44	0.09^{b,A} ± 0.06	0.23^{b,A} ± 0.17	0.18^{b,A} ± 0.13	4.98^{b,B} ± 0.23	3.83^{b,B}± 1.22	4.58^{b,B} ± 1.29	0.09^{b,A} ± 0.07	6.74^{a,B} ± 2.39	7.09^{a,C} ± 2.00
Acetic	4.41^{a} ± 2.60	0.55^{b,A} ± 0.06	8.2^{b,A} ± 0.46	9.67^{b,A} ± 0.33	6.57^{a,B} ± 2.31	5.05^{a,A} ± 3.32	5.30^{a,B} ± 1.76	0.80^{b,A} ± 0.47	21.42^{b,B} ± 0.55	35.37^{b,C} ± 5.84
Propionic	5.66^{a} ± 1.78	1.53^{b,A} ± 0.05	2.58^{b,A} ±0.38	2.34^{b,A} ± 0.26	7.09^{a,B} ± 1.57	7.17^{a,B} ± 2.38	5.10^{a,B} ± 2.20	1.06^{b,A} ± 0.38	1.22^{b,A} ± 0.44	16.61^{b,C} ± 4,74
Isobutyric	4.06^{a} ± 1.52	nd	nd	nd	13.95^{b} ± 2.64	13.80^{b} ± 4.23	9.97^{b} ± 2.23	nd	nd	nd
Butyric	0.83^{a} ± 0.64	0.82^{a,A} ± 0.32	1.25^{a,A} ± 0.11	0.79^{a,A} ± 0.18	1.08^{a,A} ± 0.21	2.66^{b,A} ± 0.97	3.31^{b,B} ± 0.14	2.34^{a,B} ± 0.92	9.65^{b,B} ± 0.86	18.23^{b,C} ± 5.62
Isovaleric	nd	nd	0.18^{a,A} ± 0.13	0.54^{a,A} ± 0.25	nd	2.01^{b,B} ± 0.33	2.58^{b,B} ± 1.30	nd	nd	0.18^{a,A} ± 0.13
Valeric	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	**27.94^{a} ± 9.98**	**2.99^{b,A} ± 0.49**	**12.44^{b,A} ± 1.25**	**13.52^{b,A} ± 1.15**	**33.67^{b,B} ± 10.96**	**34.52^{b,B} ± 5.45**	**30.84^{a,B} ± 8.92**	**4.41^{b,A} ± 1.91**	**66.43^{b,C} ± 21.82**	**93.66^{b,C} ± 21.82**

Mean ± standard deviation. nd: not detected (LOD = 0.016–0.03 mM). Lowercase letters indicate significant differences between different substrate fermentation times compared to baseline. Capital letters indicate significant differences between the different substrates at the same times.

which is very similar to the values reported for Choudhary et al. (2023) and Pangestuti et al. (2021) for *U. lactuca*, namely 6% and 7.6%, respectively. The low lipid content in this study (0.2%) is also similar to those studies (1% and 0.13%, respectively). The major component of *U. lactuca* was carbohydrates, accounting for 58.57% of the total dry weight. Similarly, Choudhary et al. (2023) and Pangestuti et al. (2021) reported a high carbohydrate content (56% and 61.83%, respectively). Carbohydrates, including polysaccharides and dietary fibre, are the main components of seaweed that have received the most attention for their ability to benefit human health (Lopez-Santamarina et al., 2020). It should be noted that the fibre content of *U. lactuca* in the present study (34.45%) is greater than that reported for terrestrial plants (30 %) (Carpena et al., 2021). Therefore, *U. lactuca* could be sold in the European Union with the claim of a 'high content of dietary fibre' (>3 g/100 g), as established by European regulation (EC) 1924/2006. This availability might encourage consumer interest in the consumption of this seaweed.

With respect to mineral composition (Table 1), the Ca and Fe contents were high (862 and 45 mg/100 g, respectively). This is notable since these minerals are essential for human growth and development, as well as for the metabolic and enzymatic reactions that take place within the organism. In addition, these elements are vital to the metabolism of the GM (Alagawany et al., 2020; Sizentsov et al., 2019). These results align with those obtained in another study conducted on *U. lactuca*: Debbarma et al. (2016), reported a Ca content of 223 mg/100 g and an Fe content of 65 mg/100 g.

After simulation of total gastrointestinal digestion (Table 1), the nutritional composition of *U. lactuca* varied due to the action of digestive enzymes and the pH. The carbohydrate and dietary fibre levels increase after digestion. This is notable because dietary fibre is the main component that is consumed by the GM (Chen et al., 2018). With respect to the mineral composition, there was an increase in Ca, Fe and Zn after digestion in the upper gastrointestinal tract, consistent with what we observed in our previous work with seaweed (Lopez-Santamarina et al., 2023).

3.2. 16S ribosomal RNA amplicon sequencing

The analysis of 16s RNA gene sequencing allows us to evaluate the evolution of the alpha and beta diversity of the intestinal microbiota at different times. It also allows to see the behaviour of the different phyla, genera, families and bacterial species present in the intestinal microbiota at different times. In addition to this, it also allows to know the metabolic pathways associated with the identified ASV.

The results obtained via 16S rRNA amplicon sequencing revealed a total of 231 ASVs. Analysis of α- and β-diversity revealed significant differences among the samples (Fig. 1a and b, respectively). Regarding the α-diversity, the graphic showed significant differences comparing the baseline (0 h) with the negative control and both substrates at all times of assay, as expected in an *in vitro* study since microbiota at time 0 h needs a period of stabilization to the basal medium (Vázquez-Rodríguez et al., 2021). There were also significant differences when comparing the negative control, inulin (positive control), and *U. lactuca* after fermentation for 24 h, with greater diversity in the case of inulin. In other work conducted with polysaccharides extracted from a brown seaweed (*Silvetia compressa*), there was an increase in diversity, albeit at longer incubation times (24 and 48 h) (Vázquez-Rodríguez et al., 2021). In terms of β-diversity, the samples at 0 h were clearly different compared with the other samples.

Regarding the taxonomy, results are shown in Fig. 2, with the relative abundance at the phylum (Fig. 2a), family (Fig. 2b), genus (Fig. 2c) and specie (Fig. 2d) levels. Regarding the different bacterial phyla (Fig. 2a), at time 0, the predominant phylum was Firmicutes, whereas after fermentation for 10, 24 and 48 h, the major phylum was Proteobacteria for all samples (the negative control, inulin and *U. lactuca*). The same trend occurred in a previous study with the brown seaweed *Himanthalia elongata* (Lopez-Santamarina et al., 2022), and a study with polysaccharides extracted from another green seaweed (*Ulva rigida*), a red seaweed (*Gracilaria fisheri*) and a brown seaweed (*Silvetia compressa*) (Charoensiddhi et al., 2022; Vázquez-Rodríguez et al., 2021). After increasing from 0 to 24 h, the relative abundance of Proteobacteria phylum decreased in all samples. In contrast, the relative abundance of the Bacteroidetes phylum decreased from 0 to 24 h and then increased at 48 h. The Firmicutes to Bacteroidetes ratio was lower for the *U. lactuca* and inulin samples compared with the negative control or the samples from 0 h. The same phenomenon occurred in another study performed with the *U. rigida* (Charoensiddhi et al., 2022).

In the case of Actinobacteria, there was a greater increase after fermentation of *U. lactuca* or inulin for 10 h compared with the negative control which showed a less notable increase (Salminen et al., 2021). The most representative families were *Enterobacteriaceae,* followed by *Ruminococcaceae* and *Lachnospiraceae* (Fig. 2b). The *Enterobacteriaceae* family can include pathogenic bacterial genera and species. Although the *Enterobacteriaceae* family was most abundant in all samples, it was relatively less extent in the *U. lactuca* and inulin samples compared with than in the negative control. There was a lower relative abundance of *Veillonellaceae* after fermentation of *U. lactuca* for 48 h compared with the positive control (inulin).

An increased abundance of *Veillonellaceae* has been reported in patients with inflammatory bowel disease and cirrhosis, suggesting that

this family has proinflammatory activities (Shukla et al., 2015). These results coincide with those found by Charoensiddhi et al. (2022) involving two green seaweeds. The increased relative abundance of *Clostridiaceae* only after fermentations of *U. lactuca* for 24 and 48 h is noteworthy. In a previous cohort study, a higher intake of terrestrial vegetables and olive oil was associated with greater relative abundance of *Clostridiaceae* in healthy humans (Castonguay-Paradis et al., 2023).

The predominant bacterial genera (Fig. 2c) were *Bacteroides* and *Parabacteroides*. This finding is consistent with previous work performed with brown seaweed (Charoensiddhi et al., 2022; Lopez-Santamarina et al., 2022). There was an increase in *Blautia* after fermentation of *U. lactuca* for 10 h and after fermentation of inulin for 48 h, unlike in the negative control. *Blautia* dominance in the GM is related to inflammatory diseases. However, the possible probiotic effects of this genus are being investigated (Mora-Flores et al., 2023). For example, different researches have reported a significant negative relationship between *Blautia* and visceral fat accumulation, regardless of sex (Mao et al., 2024; Ozato et al., 2019). The abundance of *Blautia* in the GM has also been shown to be reduced in patients with type I and II diabetes (Inoue et al., 2017; Murri et al., 2013). In addition, *Blautia* species are known to produce butyric and acetic acids, which have shown beneficial effects against obesity (Ozato et al., 2019). In addition, in an *in vitro* assay develop by Ma et al. (2023) were teste the effect of polysaccharides extracted from a green seaweed (*Enteromorpha clathrata*) on the GM, there was an increase in *Blautia* associated with an anti-cholitic effect. A low relative abundance of *Dialister*, a genus considered a psychobiotic, was found in patients with major depressive disorder, which is closely related to clinical symptoms (Yu et al., 2023).

We identified 39 species (Fig. 2d); note that "not_identified" species represent the most abundant group. Among those identified, the ten more abundant were *Parabacteroides distasonis*, *Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis, Bacteroides ovatus, Prevotella copri, Bacteroides uniformis, Bacteroides plebeius, Bacteroides coprophilus and Roseburia faecis*. Regarding the statistical analysis, there were no significant differences among the samples for the 231 identified ASVs. Among the 39 identified species, there were no significant differences among the samples, although there were trends for differences in *P. distasonis*, *B. adolescentis*, *B. ovatus*, and *P. copri* (Fig. 3). *P. distasonis* increased after fermentation of inulin or *U. lactuca* for 24 and 48 h compared with negative control (Fig. 3a), with greater abundance in the presence of inulin. This bacterial species is known to have beneficial effects on obesity, insulin sensitivity and other inflammatory processes (Abuqwider et al., 2023). *B. ovatus* also showed increased abundance after fermentation of inulin or *U. lactuca*, although only at the 48 h time point (Fig. 3b). The role of *B. ovatus* in the intestine in not determined but several authors have reported that different strains, including ELH-B2 (Tan et al., 2018), could act as probiotics. These results for *B. ovatus* are similar to those obtained in previous work with the brown seaweeds *S. compressa* and *H. elongata* (Charoensiddhi et al., 2022; Lopez-Santamarina et al., 2023). The results agree with other *in vitro* assay conducted with polysaccharides extracted from a green seaweed (*E. clathrata*), who showed a significant increase in the abundance of anti-cholitics bacteria, including *B. ovatus* and *Parabacteroides* spp. in the gut microbiota (Ma et al., 2023).

P. copri showed a trend for increased abundance after fermentation of inulin for 10 h and after fermentation of *U. lactuca* for 48 h (Fig. 3c). *P. copri* is related with consumption of fibre (Abdelsalam et al., 2023), so their abundance is associated to a health status. Finally, *B. adolescentis* showed similar changes for all samples; however, when considering the same fermentation times, the average relative abundance was higher in the presence of *U. lactuca* compared with inulin and the negative control (Fig. 3d). *B. adolescentis* is a well-known probiotic due to its beneficial effects on the health of the host (Roca-Saavedra et al., 2018) and a key member of the human gut microbiota (Duranti et al., 2020), however with these substrates its relative abundance decrease with fermentation time, indicating that, probably it do not possess the enzyme necessary to degrade the substrates used in this work.

The metabolic pathways associated with the obtained ASVs were also analysed (Fig. 4). A total of 123 metabolic pathways were identified. When analyzing the results for the fermentation of inulin, *U. lactuca*, and the negative control at 10, 24, and 48 h, 12 metabolic pathways showed significant differences (Table 2). They were increased with fermentation of *U. lactuca,* except for PWY-5304 and PWY-6572, which were increased with fermentation of inulin (Fig. 4a and b). Among the pathways increased with fermentation of *U. lactuca* are those related with the biosynthesis of vitamin B12 (P381-PWY and PWY-5507) and that related with tyrosine degradation (TYRFUMCAT-PWY), deficiencies of enzymes in this pathway are associated with several types of tyrosinemia. Fig. 4c and d shows the graphics obtained from the statistical analysis associated to these pathways.

3.3. Analysis of short-chain fatty acids

Short-chain fatty acids analysis shows the concentration of lactic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids after fermentation at different times of inulin, *U. lactuca* and negative control.

The SCFAs results are shown in Table 3. There was an increase in the total SCFA content after fermentation of *U. lactuca* or inulin for 10, 24 and 48 h compared with the negative control and the 0 h samples.

The maximum increased for total SCFAs occurred after fermentation of *U. lactuca* for 24 h and after fermentation of inulin for 48 h. These results are consistent with those observed in previous works develop by our research group performed with the seaweeds *H. elongata* and *U. pinnatifida* (Lopez-Santamarina et al., 2022, 2023). Regarding individual SCFAs there was a decrease in lactic acid in all samples after fermentation compared with 0 h, and major increases in propionic, butyric, isobutyric and isovaleric acids. The levels of these SCFAs increased, although with different trends Depending on whether the carbon source was *U. lactuca* or inulin.

Propionic acid increased after fermentation of *U. lactuca* at all times compared with the negative control. Only fermentation of inulin for 48 h increased propionic acid. This SCFA has been shown to have antilipogenic and cholesterol-lowering effects. It also elicits strong effects towards weight control and feeding behaviour. Furthermore, there is evidence that propionate exerts, just as butyrate, an antiproliferative effect towards colon cancer cells (Hosseini et al., 2011).

The major production of butyric acid was observed for the assay with inulin at 48 h of fermentation, whereas isobutyric acid concentration was higher in *U. lactuca* assays after 24 h. These SCFAs inhibit of proinflammatory cytokine production and possess anti-inflammatory properties (Sun et al., 2023). Butyric acid also promotes the absorption of calcium, iron and magnesium (Zhang et al., 2023).

Finally, fermentation of *U. lactuca,* but not inulin, increase isovaleric acid. This finding is important because isovaleric acid was correlated with a variety of neurotransmitters (Zhong et al., 2023).

4. Conclusions

This is the first *in vitro* study on the potential modulation of human gut microbiota by a whole green seaweed, *U. lactuca,* which is rich in fibre and Ca, and thus may be useful as a supplement for diets deficient in these nutrients. When compared with inulin regarding the potential to modulate the GM, there were no significant differences in the obtained ASVs. Regarding the metabolic pathways related with the GM functions, we identified 12 pathways with significant differences, 10 of them increased with fermentation of *U. lactuca* and 2 of them increased with fermentation of inulin. Among the pathways increased due to fermentation of *U. lactuca*, two are related with the biosynthesis of cobalamin (vitamin B12) and with the thyrosine degradation or nitrate reduction.

We found significant differences in SCFAs during fermentation Although fermentation of inulin showed higher production of these acids, especially acetic, propionic, and butyric acids, fermentation of

U. lactuca also increased them compared with the negative control and the 0 h samples. Moreover, the highest isovaleric acid level occurred after fermentation of *U. lactuca*.

Although more studies are needed to validate these results, this work shows for the first time as *U. lactuca* could modulate the human gut microbiota and provide some benefits for the human health.

CRediT authorship contribution statement

Aroa López-Santamarina: Writing – original draft, Methodology, Investigation. **Paula Roade-Pérez:** Writing – original draft, Methodology, Investigation. **Alicia del Carmen Mondragón-Portocarrero:** Writing – review & editing, Supervision. **Alejandra Cardelle-Cobas:** Supervision, Investigation, Formal analysis. **Alberto Cepeda:** Investigation, Funding acquisition. **José Manuel Miranda:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Aroa Lopez-Santamarina is grateful for a predoctoral grant funded by the Universidade de Santiago de Compostela (USC), Campus Terra, Lugo, (Contratos predoutorais do campus de especialización Campus Terra).

Data availability

Data will be made available on request.

References

Abdelsalam, N. A., Hegazy, S. M., & Aziz, R. K. (2023). The curious case of *Prevotella copri*. *Gut Microbes, 15*(2), Article 2249152. https://doi.org/10.1080/19490976.2023.2249152

Abuqwider, J., Di Porzio, A., Barrella, V., Gatto, C., Sequino, G., De Filippis, F., Crescenzo, R., Spagnuolo, M. S., Cigliano, L., Mauriello, G., Iossa, S., & Mazzoli, A. (2023). *Limosilactobacillus reuteri* DSM 17938 reverses gut metabolic dysfunction induced by Western diet in adult rats. *Frontiers in Nutrition, 10*, Article 1236417. https://doi.org/10.3389/fnut.2023.1236417

Ajanth Praveen, M., Karthika Parvathy, K. R., Jayabalan, R., & Balasubramanian, P. (2019). Dietary fiber from Indian edible seaweeds and its *in-vitro* prebiotic effect on the gut microbiota. *Food Hydrocolloids, 96*, 343–353. https://doi.org/10.1016/j.foodhyd.2019.05.031

Alagawany, M., Elnesr, S. S., Farag, M. R., Tiwari, R., Yatoo, M. I., Karthik, K., Michalak, I., & Dhama, K. (2020). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. *Veterinary Quarterly, 41*(1), 1–29. https://doi.org/10.1080/01652176.2020.1857887

Biancarosa, I., Belghit, I., Bruckner, C. G., Liland, N. S., Waagbø, R., Amlund, H., Heesch, S., & Lock, E. J. (2018). Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: Benefits of and limitations to their potential use in food and feed. *Journal of the Science of Food and Agriculture, 98*(5), 2035–2042. https://doi.org/10.1002/jsfa.8798

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Carpena, M., Caleja, C., Pereira, E., Pereira, C., Ćirić, A., Soković, M., Soria-Lopez, A., Fraga-Corral, M., Simal-Gandara, J., Ferreira, I. C. F. R., Barros, L., & Prieto, M. A. (2021). Red seaweeds as a source of nutrients and bioactive compounds: Optimization of the extraction. *Chemosensors, 9*(6), 132. https://doi.org/10.3390/chemosensors9060132

Castonguay-Paradis, S., Perron, J., Flamand, N., Lamarche, B., Raymond, F., Di Marzo, V., & Veilleux, A. (2023). Dietary food patterns as determinants of the gut microbiome–endocannabinoidome axis in humans. *Scientific Reports, 13*(1), Article 15702. https://doi.org/10.1038/s41598-023-41650-z

Charoensiddhi, S., Conlon, M., Methacanon, P., Thayanukul, P., Hongsprabhas, P., & Zhang, W. (2022). Gut microbiome modulation and gastrointestinal digestibility in vitro of polysaccharide-enriched extracts and seaweeds from *Ulva rigida* and *Gracilaria fisheri*. *Journal of Functional Foods, 96*, Article 105204. https://doi.org/10.1016/j.jff.2022.105204

Charoensiddhi, S., Conlon, M. A., Vuaram, M. S., Franco, C. M. M., & Zhanf, W. (2016). Impact of extraction processes on prebiotic potential of the brown seaweed *Ecklonia radiata* by *in vitro* human gut bacteria fermentation. *Journal of Functional Foods, 24*(4), 221–230. https://doi.org/10.1016/j.jff.2016.04.016

Chen, G., Xie, M., Wan, P., Chen, D., Ye, H., Chen, L., Zeng, X., & Liu, Z. (2018). Digestion under saliva, simulated gastric and small intestinal conditions and fermentation *in vitro* by human intestinal microbiota of polysaccharides from Fuzhuan brick tea. *Food Chemistry, 244*, 331–339. https://doi.org/10.1016/j.foodchem.2017.10.074

Choudhary, B., Khandwal, D., Gupta, N. K., Patel, J., & Mishra, A. (2023). Nutrient composition, physicobiochemical analyses, oxidative stability and antinutritional assessment of abundant tropical seaweeds from the arabian sea. *Plants, 12*(12), 2302. https://doi.org/10.3390/plants12122302

Cosgrove, W. J., & Loucks, D. P. (2015). Water management: Currentand future challenges and researchdirections. *Water Resources Research, 51*, 4823–4839. https://doi.org/10.1002/2014WR016869

Debbarma, J., Madhusudana Rao, B., Narasimha Murthy, L., Mathew, S., Venkateshwarlu, G., & Ravishankar, C. N. (2016). Nutritional profiling of the edible seaweeds *Gracilaria edulis, Ulva lactuca* and *Sargassum* sp. *Indian Journal of Fisheries, 63*(3), 81–87. https://doi.org/10.21077/ijf.2016.63.3.60073-11

Degruttola, A. K., Low, D., Mizoguchi, A., & Mizoguchi, E. (2016). Current understanding of dysbiosis in disease in human and animal models. *Inflammatory Bowel Diseases, 22*(5), 1137–1150. https://doi.org/10.1097/MIB.0000000000000750

Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology, 38*(6), 685–688. https://doi.org/10.1038/s41587-020-0548-6. Nature Research.

Duranti, S., Ruiz, L., Lugli, G. A., Tames, H., Milani, C., Mancabelli, L., Mancino, W., Longhi, G., Carnevali, L., Sgoifo, A., Margolles, A., Ventura, M., Ruas-Madiedo, P., & Turroni, F. (2020). *Bifidobacterium adolescentis* as a key member of the human gut microbiota in the production of GABA. *Scientific Reports, 10*(1), Article 14112. https://doi.org/10.1038/s41598-020-70986-z

El-Beltagi, H. S., Mohamed, A. A., Mohamed, H. I., Ramadan, K. M. A., Barqawi, A. A., & Mansour, A. T. (2022). Phytochemical and potential properties of seaweeds and their recent applications: A review. *Marine Drugs, 20*(6), 342. https://doi.org/10.3390/md20060342. MDPI.

Fang, Q., Hu, J., Nie, Q., & Nie, S. (2019). Effects of polysaccharides on glycometabolism based on gut microbiota alteration. *Trends in Food Science & Technology, 92*, 65–70. https://doi.org/10.1016/j.tifs.2019.08.015

Gade, A., & Kumar, M. S. (2023). Gut microbial metabolites of dietary polyphenols and their potential role in human health and diseases. *Journal of Physiology & Biochemistry, 79*(4), 695–718. https://doi.org/10.1007/s13105-023-00981-1

Hosseini, E., Grootaert, C., Verstraete, W., & Van de Wiele, T. (2011). Propionate as a health-promoting microbial metabolite in the human gut. *Nutrition Reviews, 69*(5), 245–258. https://doi.org/10.1111/j.1753-4887.2011.00388.x

Inoue, R., Ohueekitano, R., Tsukahara, T., Tanaka, M., Masuda, S., Inoue, T., Yamakage, H., Kusakabe, T., Hasegawa, K., Shimatsu, A., & Satohhasahara, N. (2017). Prediction of functional profiles of gut microbiota from 16S rRNA metagenomic data provides a more robust evaluation of gut dysbiosis occurring in Japanese type 2 diabetic patients. *Journal of Clinical Biochemistry & Nutrition, 61*(3), 217–221. https://doi.org/10.3164/jcbn.17744

Kansandee, W., Moonmangmee, S., Vangpikul, S., Kosawatpat, P., & Tamtin, M. (2024). Physicochemical properties and *in vitro* prebiotic activity of *Ulva rigida* polysaccharides. *Biocatalysis and Agricultural Biotechnology, 59*, Article 103252. https://doi.org/10.1016/j.bcab.2024.103252

Kong, Q., Dong, S., Gao, J., & Jiang, C. (2016). *In vitro* fermentation of sulfated polysaccharides from *E. prolifera* and *L. japonica* by human fecal microbiota. *International Journal of Biological Macromolecules, 91*, 867–871. https://doi.org/10.1016/j.ijbiomac.2016.06.036

Lopez-Santamarina, A., Cardelle-Cobas, A., del Carmen Mondragon, A., Sinisterra-Loaiza, L., Miranda, J. M., & Cepeda, A. (2022). Evaluation of the potential prebiotic effect of *Himanthalia elongata*, an Atlantic brown seaweed, in an *in vitro* model of the human distal colon. *Food Research International, 156*, Article 111156. https://doi.org/10.1016/j.foodres.2022.111156

Lopez-Santamarina, A., Miranda, J. M., Del Carmen Mondragon, A., Lamas, A., Cardelle-Cobas, A., Franco, C. M., & Cepeda, A. (2020). Potential use of marine seaweeds as prebiotics: A review. *Molecules, 25*(4), 1004. https://doi.org/10.3390/molecules25041004

Lopez-Santamarina, A., Sinisterra-Loaiza, L., Mondragón-Portocarrero, A., Ortiz-Viedma, J., Cardelle-Cobas, A., Abuín, C. M. F., & Cepeda, A. (2023). Potential prebiotic effect of two Atlantic whole brown seaweeds, *Saccharina japonica* and *Undaria pinnatifida*, using in vitro simulation of distal colonic fermentation. *Frontiers in Nutrition, 10*, Article 1170392. https://doi.org/10.3389/fnut.2023.1170392

Lozano Muñoz, I., & Díaz, N. F. (2020). Minerals in edible seaweed: Health benefits and food safety issues. *Critical Reviews in Food Science and Nutrition, 62*(6), 1592–1607. https://doi.org/10.1080/10408398.2020.1844637

Ma, M., Quan, M., Zhang, J., Zhang, A., Gao, P., Shang, Q., & Yu, G. (2023). *In vitro* fermentation of polysaccharide from edible alga *Enteromorpha clathrata* by the gut microbiota of patients with ulcerative colitis. *Nutrients, 15*(19), 4122. https://doi.org/10.3390/nu15194122

Mahendran, S., Maheswari, P., Sasikala, V., Rubika, J. jaya, & Pandiarajan, J. (2021). In vitro antioxidant study of polyphenol from red seaweeds dichotomously branched gracilaria *Gracilaria edulis* and robust sea moss *Hypnea valentiae*. *Toxicology Reports, 8*, 1404–1411. https://doi.org/10.1016/j.toxrep.2021.07.006

Mao, B., Guo, W., Cui, S., Zhang, Q., Zhao, J., Tang, X., & Zhang, H. (2024). Blautia producta displays potential probiotic properties against dextran sulfate sodium-induced colitis in mice. *Food Science and Human Wellness, 13*(2), 709–720. https://doi.org/10.26599/FSHW.2022.9250060

Miao, X., Xiao, J., Xu, Q., Fan, S., Wang, Z., Wang, X., & Zhang, X. (2020). Distribution and species diversity of the floating green macroalgae and micro-propagules in the Subei Shoal, southwestern Yellow Sea. *PeerJ, 8*, Article e10538. https://doi.org/10.7717/peerj.10538

Mora-Flores, L. P., Moreno-Terrazas Casildo, R., Fuentes-Cabrera, J., Pérez-Vicente, H. A., de Anda-Jáuregui, G., & Neri-Torres, E. E. (2023). The role of carbohydrate intake on the gut microbiome: A weight of evidence systematic review. *Microorganisms, 11*(7), 1728. https://doi.org/10.3390/microorganisms11071728

Murri, M., Leiva, I., Gomez-Zumaquero, J. M., Tinahones, F. J., Cardona, F., Soriguer, F., & Queipo-Ortuño, M. I. (2013). Gut microbiota in children with type 1 diabetes differs from that in healthy children: A case-control study. *BMC Medicine, 11*(1), 46. https://doi.org/10.1186/1741-7015-11-46

Ozato, N., Saito, S., Yamaguchi, T., Katashima, M., Tokuda, I., Sawada, K., Katsuragi, Y., Kakuta, M., Imoto, S., Ihara, K., & Nakaji, S. (2019). *Blautia* genus associated with visceral fat accumulation in adults 20–76 years of age. *Npj Biofilms and Microbiomes, 5*(1), 28. https://doi.org/10.1038/s41522-019-0101-x

Pangestuti, R., Haq, M., Rahmadi, P., & Chun, B. S. (2021). Nutritional value and biofunctionalities of two edible green seaweeds (*Ulva lactuca* and *caulerpa racemosa*) from Indonesia by subcritical water hydrolysis. *Marine Drugs, 19*(10), 578. https://doi.org/10.3390/md19100578

Poole, J., Diop, A., Rainville, L. C., & Barnabe, S. (2019). Bioextracting polyphenols from the Brown seaweed *Ascophyllum nodosum* from québec's north shore coastline. *Industrial Biotechnology, 15*(3), 212–218. https://doi.org/10.1089/ind.2019.0008

Rasyid, A. (2017). Evaluation of nutritional composition of the dried seaweed *Ulva lactuca* from Pameungpeuk waters, Indonesia. *Tropical Life Sciences Research, 28*(2), 119–125. https://doi.org/10.21315/tlsr2017.28.2.9

Ren, X., Liu, L., Gamallat, Y., Zhang, B., & Xin, Y. (2017). Enteromorpha and polysaccharides from enteromorpha ameliorate loperamide-induced constipation in mice. *Biomedicine & Pharmacotherapy, 96*, 1075–1081. https://doi.org/10.1016/j.biopha.2017.11.119

Roca-Saavedra, P., Mendez-Vilabrille, V., Miranda, J. M., Nebot, C., Cardelle-Cobas, A., Franco, C. M., & Cepeda, A. (2018). Food additives, contaminants and other minor components: Effects on human gut microbiota—a review. *Journal of Physiology & Biochemistry, 74*(1), 69–83. https://doi.org/10.1007/s13105-017-0564-2

Rolhion, N., & Chassaing, B. (2016). When pathogenic bacteria meet the intestinal microbiota. *Philosophical Transactions of the Royal Society of London B Biological Sciences, 371*(1707), Article 20150504. https://doi.org/10.1098/rstb.2015.0504

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition, 57*(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8

Seong, H., Bae, J. H., Seo, J. S., Kim, S. A., Kim, T. J., & Han, N. S. (2019). Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using *in vitro* human fecal fermentation. *Journal of Functional Foods, 57*, 408–416. https://doi.org/10.1016/j.jff.2019.04.014

Shang, Q., Wang, Y., Pan, L., Niu, Q., Li, C., Jiang, H., Cai, C., Hao, J., Li, G., & Yu, G. (2018). Dietary polysaccharide from *Enteromorpha clathrata* modulates gut microbiota and promotes the growth of *Akkermansia muciniphila, Bifidobacterium* spp. and *Lactobacillus* spp. *Marine Drugs, 16*(5), 167. https://doi.org/10.3390/md16050167

Shukla, R., Ghoshal, U., Dhole, T. N., & Ghoshal, U. C. (2015). Fecal microbiota in patients with irritable bowel syndrome compared with healthy controls using real-time polymerase chain reaction: An evidence of dysbiosis. *Digestive Diseases and Sciences, 60*(10), 2953–2962. https://doi.org/10.1007/s10620-015-3607-y

Sizentsov, A., Sizentsov, Y., Kvan, O., Salnikova, E., & Salnikova, V. (2019). A study on heavy metal sorption properties of intestinal microbiota *in vitro*. *E3S Web of Conferences, 79*, Article 03021. https://doi.org/10.1051/e3sconf/20197903021

Sun, J., Chen, J., Xie, Q., Sun, M., Zhang, W., Wang, H., Liu, N., Wang, Q., & Wang, M. (2023). Sodium butyrate alleviates R97-116 peptide-induced myasthenia gravis in mice by improving the gut microbiota and modulating immune response. *Journal of Inflammation, 20*(1), 37. https://doi.org/10.1186/s12950-023-00363-w

Torres, M. D., Flórez-Fernández, N., & Domínguez, H. (2019). Integral utilization of red seaweed for bioactive production. *Marine Drugs, 17*(6). https://doi.org/10.3390/md17060314

Vázquez-Rodríguez, B., Santos-Zea, L., Heredia-Olea, E., Acevedo-Pacheco, L., Santacruz, A., Gutiérrez-Uribe, J. A., & Cruz-Suárez, L. E. (2021). Effects of phlorotannin and polysaccharide fractions of brown seaweed *Silvetia compressa* on human gut microbiota composition using an *in vitro* colonic model. *Journal of Functional Foods, 84*, Article 104596. https://doi.org/10.1016/j.jff.2021.104596

Wekre, M. E., Kåsin, K., Underhaug, J., Jordheim, M., & Holmelid, B. (2019). Quantification of polyphenols in seaweeds: A case study of *Ulva intestinalis*. *Antioxidants, 8*(12), 612. https://doi.org/10.3390/antiox8120612

Yan, X., Yang, C., Lin, G., Chen, Y., Miao, S., Liu, B., & Zhao, C. (2019). Antidiabetic potential of green seaweed *Enteromorpha prolifera* flavonoids regulating insulin signaling pathway and gut microbiota in type 2 diabetic mice. *Journal of Food Science, 84*(1), 165–173. https://doi.org/10.1111/1750-3841.14415

Yu, S., Wang, L., Jing, X., Wang, Y., & An, C. (2023). Features of gut microbiota and short-chain fatty acids in patients with first-episode depression and their relationship with the clinical symptoms. *Frontiers in Psychology, 14*, Article 1088268. https://doi.org/10.3389/fpsyg.2023.1088268

Zhang, X., Aweya, J. J., Huang, Z. X., Kang, Z. Y., Bai, Z. H., Li, K. H., He, X. T., Liu, Y., Chen, X. Q., & Cheong, K. L. (2020). *In vitro* fermentation of *Gracilaria lemaneiformis* sulfated polysaccharides and its agaro-oligosaccharides by human fecal inocula and its impact on microbiota. *Carbohydrate Polymers, 234*, Article 115894. https://doi.org/10.1016/j.carbpol.2020.115894

Zhang, X., Li, N., Wang, G., Zhang, C., Zhang, Y., Zeng, F., Liu, H., Yi, G., & Wang, Z. (2023). Research status of polysiloxane-based piezoresistive flexible human electronic sensors. *RSC Advances, 13*(24), 16693–16711. https://doi.org/10.1039/d3ra03258b

Zhong, J. G., Lan, W. T., Feng, Y. Q., Li, Y. H., Shen, Y. Y., Gong, J. H., Zou, Z., & Hou, X. (2023). Associations between dysbiosis gut microbiota and changes of neurotransmitters and short-chain fatty acids in valproic acid model rats. *Frontiers in Physiology, 14*, Article 107821. https://doi.org/10.3389/fphys.2023.1077821

Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: Diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology, 16*(1), 35–56. https://doi.org/10.1038/s41575-018-0061-2